Clinical Study Protocol

Drug Substance	NXL104
Study Code	D4280C00008
Edition Number	1
Date	

An Open Label Single-Dose Study in Healthy Male Subjects Designed to Assess the Mass Balance Recovery, Metabolite profile and Metabolite Identification of [^{14}C]NXL104

Sponsor:

The following Amendment(s) and Administrative Changes have been made to this protocol since the date of preparation:

Amendment No.	Date of Amendment	Local Amendment No:	Date of Local Amendment

Administrative Change No.	Date of Administrative Change	Local Administrative Change No.	Date of Local Administrative Change

AstraZeneca

PROTOCOL SYNOPSIS

An Open Label Non-randomised Single-Dose Study in Healthy Subjects Designed to Assess the metabolism and elimination of [14C]NXL104 following a single IV dose of 500 mg

Principal Investigator

Study centre(s) and number of subjects planned

Six healthy male subjects will be enrolled

Study period	Phase of development
Estimated date of first subject enrolled	Phase I
Estimated date of last subject completed	

Objectives

The primary objectives of the study are:

- To determine the mass balance after a single intravenous (IV) dose of [^{14}C]NXL104
- To determine the routes of [^{14}C]NXL104 metabolism and excretion
- To estimate the whole blood and plasma partitioning of total radioactivity
- To determine the urine and faecal recovery of radioactivity

The secondary objectives of the study are:

- To assess the IV pharmacokinetics (PK) of [^{14}C]NXL104
- To identify and characterise the metabolites of [^{14}C]NXL104 in plasma, whole blood, urine and faeces
- To provide additional safety and tolerability information for NXL104

Study design

This is an open-label, single dose, mass balance, metabolite profiling and identification study performed in performed in six healthy subjects. Each subject will receive a single IV infusion administration of approximately 500 mg NXL104 in approximately 100 mL delivered over approximately 60 minutes containing a target radiation dose of but not more than (NMT) 300 μCi (11.1 MBq) [^{14}C]NXL104 in the fasted state.

Target subject population

Healthy male subjects aged 30-65 years with a body mass index (BMI) between 18 and 32 kg/m^2 inclusive

Investigational product, dosage and mode of administration

Single IV dose of 500 mg [^{14}C]NXL104

Comparator, dosage and mode of administration

Not Applicable

Duration of treatment

Each subject will receive a single IV infusion administered over approximately 60 minutes.

Outcome variable(s):

- Pharmacokinetics

  Maximum concentrations (C_{max}), time to C_{max} (t_{max}), area under the concentration-time curve extrapolated to infinity ($AUC_{0-\infty}$), terminal elimination rate constant (λ_z), terminal elimination half life ($t_{1/2}$), volume of distribution at steady state (V_{ss}) and mean residence time (MRT) will be estimated for total radioactivity (in plasma and whole blood) and NXL104 (in plasma).

  The amount of NXL104 excreted (Ae), % excreted will be calculated from NXL104 concentrations in urine and faeces. Renal clearance (CLr) and Total clearance (CL_T) will be calculated from NXL104 concentrations in urine.

  PK parameters for metabolites of NXL104 will be calculated based on plasma, urine and faces concentration levels. Identification of major metabolites in plasma, urine and faeces will be performed where possible.

  Mass Balance and cumulative recovery of total radioactivity in urine, faeces & total (urine and faeces) will be generated.

  All concentrations below the limit of quantification and/or missing will be labelled as such. Any subjects and samples excluded will be clearly documented in the study report.

- Safety

  Adverse events, clinical chemistry, haematology, urinalysis, vital signs, electrocardiogram (ECG), physical examination

- Statistical methods

  No formal statistical hypothesis testing will be performed. For the analyses of safety, tolerability and pharmacokinetic data descriptive statistics including n, mean, standard deviation (SD), median, minimum, maximum and coefficient of variance (CV%) will be presented in summary tables, figures and data listings.

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

The following abbreviations and special terms are used in this study Clinical Study Protocol.

Abbreviation or special term	Explanation
Ae	Amount of drug excreted
AE	Adverse event (see definition in Section 6.3.1)
ALT	Alanine Transferase
ALP	Alanine phosphatase
AmpC	Ambler Class C
ARSAC	Administration of Radioactive Substances Advisory Committee
AST	Aspartate Transferase
AUC	Area under the curve
BMI	Body Mass Index
C_{max}	Maximum concentration
CBC	Complete blood count with differential
CL_T	Total Clearance
CL_r	Renal Clearance
CSA	Clinical Study Agreement
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Event
CV	Coefficient of Variance
DAE	Discontinuation of Investigational Product due to Adverse Event
DNA	Deoxyribonucleic acid
DUS	Disease under Study
ECG	Electrocardiogram
EC	Ethics Committee, synonymous to Institutional Review Board (IRB) and Independent Ethics Committee (IEC)

Abbreviation or special term	Explanation
eCRF	Electronic Case Report Form
EMA	European medicines Agency
GCP	Good Clinical Practice
GGTP	Gamma Glutamyl Transpeptidase
HIV	Human Immunodeficiency Virus
HPA	Health Protection Agency
IC	Inhibitory concentration
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICU	Intensive Care Unit
IP	Investigational Product
IUD	Intra-uterine Device
IV	Intravenous
LCMS	Liquid Chromatography Mass Spectroscopy
LIMS	Laboratory Information Management System
LLOQ	Lower Limit of Quantification
LSC	Liquid Scintillation Counter
LSLV	Last Subject Last Visit
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Corpuscular Volume
MDR	Multidrug resistant
MeDRA	Medical Dictionary for Regulatory Activities
MHRA	Medicines and Healthcare Regulatory Authority
MRT	Mean Residence Time
OAE	Other Significant Adverse Event (see definition in Section 11.1.1)
PCV	Packed cell volume
PK	Pharmacokinetic
PI	Principal Investigator
QC	Quality Control
RAP	Reporting and Analysis Plan

Abbreviation or special term	Explanation
SAE	Serious adverse event (see definition in Section 6.3.2).
RBC	Red Blood Cells
SD	Standard deviation
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected serious adverse events
t_{max}	Time taken to reach maximum concentration
$t_{1/2}$	Terminal half-life
ULN	Upper Limit of Normal
UTI	Urinary Tract infection
WBDC	Web Based Data Capture
V_{ss}	Volume of distribution at steady state
λ_z	Terminal rate constant
% excreted	% dose excreted

1. INTRODUCTION

1.1 Background

It is estimated that, in the United States alone, 2 million patients a year acquire infections while in hospital. Some 350,000 (10% to 20%) of these infections involve the bloodstream, and 90,000 (4.5%) are fatal (D'Agata 2004; Kang et al, 2003; Cosgrove et al, 2004; Karlowsky et al, 2004). Gram-negative pathogens are responsible for nosocomial infections in 42%, 46% and 63% of intensive care units (ICUs), non-ICUs, and outpatient wings, respectively (Fraser et al, 2008; Jacoby and Han 1996). Gram-negative pathogens are also responsible for a substantial proportion of infections in the community.

Among the gram-negative pathogens, Enterobacteriaceae (*Escherichia, Klebsiella*, *Enterobacter, Serratia, Proteus*, and *Citrobacter*) currently cause 29% of nosocomial infections in the United States. This group of pathogens is responsible for 46% of urinary tract infections (UTIs), 24% of surgical site infections, 17% of bacteraemia cases, and 30% of pneumonia cases (Fridkin et al, 1999).

Among community-acquired infections, *Escherichia coli* is the major cause of UTIs, including prostatitis, pyelonephritis (hospitalisation due to pyelonephritis) and septicemia. Proteus, Klebsiella, and Enterobacter species are also common urinary tract pathogens. Proteus mirabilis is the most frequent cause of infection-related kidney stones (Hiong U Go et al, 2004).

The prevalence of multidrug-resistant (MDR) strains (resistance to at least 3 different antibiotic groups) among gram negative bacilli is increasing (D'Agata 2004, Gales et al, 2001, Karlowsky et al; 2003a, Karlowsky et al, 2003b). Compared with infections due to antimicrobial-susceptible gram negative bacilli, infections due to MDR gram negative bacilli lead to longer hospital stays, increased mortality and greater costs of hospitalisation (Cosgrove et al, 2004, Lautenbach et al, 2001).

NXL104 is a novel non β-lactam β-lactamase inhibitor that is under development in combination with β-lactam antibiotics to treat infections that have become resistant to β lactam antibiotics alone. NXL104 is being co-developed by AstraZeneca, Forest and Cerexa in combination with ceftazidime (CAZ104) for the treatment of complicated urinary tract infection (cUTI) and complicated intra abdominal infection (cIAI), and with ceftaroline fosamil (CXL104) for the treatment of cUTI.

The compound has a spectrum of activity encompassing both AmpA and AmpC β-lactamases, which include enzymes of profound clinical importance. CAZ104 and CXL104 have been shown to be active against strains which express a combination of β lactamase types, as well as strains which are concomitantly resistant to other antibacterial classes such as fluoroquinolones. NXL104 inhibits β-lactamases by forming a highly stable, practically irreversible covalent binding to these enzymes with a lower half maximal inhibitory concentration (IC50) compared with the currently marketed β lactamases inhibitors clavulanic

acid, tazobactam and sulbactam. In addition, NXL104 is a potent inhibitor of class C (AmpC) enzymes whereas clavulanic acid, tazobactam, and sulbactam lack such activity. Unlike currently available β-lactamase inhibitors, NXL104 does not induce β-lactamase production in Enterobacter cloacae. Although NXL104 has virtually no intrinsic antibacterial activity, it efficiently protects β-lactams from hydrolysis by a variety of class A (AmpA) and class C (AmpC) β-lactamase–producing strains, including extended spectrum β lactamases (ESBL) producers. NXL104 will however only be developed for use in combination with another compound and will not be used as a single agent. The potent in vitro activity of CAZ104 against *Enterobacteriaceae* producing class A and, importantly, class C β-lactamases has been confirmed *in vivo* in murine pneumonia, septicemia and pyelonephritis models.

Currently, the options for the treatment of gram-negative infections, especially MDR strains including ESBL producers, are limited. Hence, the development of well tolerated combinations which have efficacy against MDR gram-negative pathogens including ESBL producers will be a significant advancement in the available treatment options for clinicians.

For further details see the NXL104 Investigator's Brochure (IB).

1.2 Rationale for conducting this study

An intravenous formulation of NXL104 is being developed as an antibacterial agent in combination with cephalosporin antibiotics for the treatment of gram negative infections, especially multidrug-resistance strains.

The specific aims of the study are to evaluate the mass balance and the pharmacokinetic profile of an intravenous formulation of NXL104. The study also aims to define the routes of elimination and the excretion kinetics of the [^{14}C]NXL104 in humans following intravenous administration, and to investigate the nature of the metabolites present in plasma and excreta.

1.3 Benefit/risk and ethical assessment

The major risk for healthy volunteers who participate in the study is from adverse events (AEs) induced by Investigational Products (IPs). There is a slight risk of infection or bruising that may occur as a result of phlebotomy. There are no direct benefits for healthy volunteers participating in the study.

Four clinical studies have been completed in subjects to assess safety and pharmacokinetics (PK) parameters, an escalating single dose study with and without ceftazidime in healthy young men with doses up to 2000 mg of NXL104, a multidose study over 5 or 10 days in healthy young men with doses up to 1000 mg every 8 hours for 5 days or 500 mg for 10 days, a single dose study to assess effect of age and gender in healthy young and elderly men and women as well as an open-label PK study in patients with mild to moderate renal impairment.

In the single dose study a total of 6 treatment-emergent AEs (TEAEs) were reported by 4 subjects. The reported events were: abdominal pain, sense of oppression, somnolence, anxiety, postural dizziness and orthostatic hypotension. There was no dose dependency in the

number of AEs or in their intensity, and all subjects recovered from the AEs without sequelae. No subject withdrew due to AEs.

Both maximum plasma concentration (C_{max}) and area under the plasma concentration time curve from zero to the time of the last measurable concentration ($AUC_{[0-t]}$) appeared to increase in direct proportion to dose within the dose range of 50 mg to 1000 mg with a slight deviation from proportion when considering the whole dose range from 20 mg to 2000 mg.

In the multiple dose study, NXL104 was generally well tolerated with no serious adverse events (SAEs) and no subject withdrew from the study due to AEs. None of the AEs observed was considered related to NXL104 treatment. No AEs were reported in the 1000 mg group.

As of August 2010, >300 healthy volunteers, patients with differing degrees of renal impairment and patients with infection, in Phase I and Phase II studies have received either NXL104 alone or CAZ104, administered by a 30-minute infusion. The dose levels for NXL104 ranged from 50 mg to 2000 mg NXL104, and combination doses of 2000 mg ceftazidime/500 mg NXL104 (healthy volunteers and patients with cIAI) and 500 mg ceftazidime/125 mg NXL104 (patients with cUTI).

A single dose study (500 mg) was conducted to evaluate the effect of age and gender on the PK and safety of NXL104 in healthy subjects. There were no SAEs or AEs leading to withdrawal from the study. Overall, 3 subjects experienced 6 TEAEs considered to be related to NXL104 treatment, including dry mouth, feeling hot, feeling jittery, dysgeusia, headache and hyperhidrosis.

In all of the Phase I studies, there were no serious or severe AEs, or AEs that led to premature withdrawal from the study. The safety profile of CAZ104 appeared to be similar to those following the individual NXL104 dose. The safety data from the Phase II study in cIAI patients showed that CAZ104 was generally well tolerated. To date, there are no serious adverse event reports associated with QT prolongation for NXL104 or CAZ104.

In order to assess the metabolism and elimination of NXL104, the dosage form in this study will contain a radionucleotide (a radiation dose of NMT 300μCi (11.1 MBq)) ^{14}C) so subjects will be exposed to ionising radiation. The nominal effective dose that each subject will receive from the single administration will not exceed 1 mSv. This maximum effective dose is comparable to the average natural background radiation received in the UK each 4.5 months (approximately 2.7 mSv received per year) or is comparable to 1-2 abdominal x-rays (approximately 0.7mSv each). It is believed that any increase in the amount of radiation that is received above natural radiation carries a risk of later developing serious and possible fatal conditions. The risk associated with the maximum possible dose of radiation in this study is very small indeed and is considered to be acceptable.

To assess tolerability of the IV solution the first volunteer will be dosed with the oral formulation at approximately 08:00. The second volunteer will be dosed approximately 30 minutes after the first volunteer. All subsequent subject dosing will be staggered by 15

minutes. It is possible that injection site reaction, bruising and other inflammation may be experienced at the site of infusion.

During cannulation, more than one attempt may be needed to insert the cannula in a vein of the subject and it is possible that bruising and/or inflammation may be experienced at the site of cannulation.

There is no benefit to the subjects from taking part in this study. The development of NXL104 may be of benefit to the wider community/patients with a variety of infections.

The overall risk-benefit balance is therefore considered to be acceptable.

For further details, see the NXL104 IB.

2. STUDY OBJECTIVES

2.1 Primary objective

1. To determine the mass balance after a single IV dose of [^{14}C]NXL104
2. To determine the routes of [^{14}C]NXL104 metabolism and excretion
3. To estimate the whole blood and plasma partitioning of total radioactivity
4. To determine the urine and faecal recovery of radioactivity

2.2 Secondary objectives

1. To assess the IV PK of [^{14}C]NXL104
2. To identify and characterise the metabolites of [14C]NXL104 in plasma, whole blood, urine and faeces
3. To provide additional safety and tolerability information for NXL104

3. STUDY PLAN AND PROCEDURES

This Clinical Study Protocol has been subject to a peer review according to AstraZeneca standard procedures.

3.1 Overall study design and flow chart

This will be an open label, non randomised, single-dose, single-centre study performed in 6 healthy male subjects designed to assess the mass balance recovery, pharmacokinetics, metabolite profile and identification following dosing of 500 mg [^{14}C]NXL104.

All subjects will receive a single IV infusion administration containing approximately 500 mg NXL104 in approximately 100 mL over approximately 60 minutes and a target dose of radioactivity of not more than 300 μCi (11.1 MBq) [^{14}C]NXL104 in a fasted state. This dose of radioactivity has been chosen to minimise the radiation dose, whilst providing sufficient [^{14}C] in blood, plasma, urine and faeces, to allow quantification of compound related material.

To assess tolerability of the IV administration, the first subject will be dosed at least 30 minutes prior to dosing the second subject. All subsequent subject dosing of the IV formulation will be staggered by at least 15 minutes.

Subjects will be invited to attend for screening which will be ≤21 days prior to Visit 2. At Visit 2, subjects will be admitted to the clinical unit on the morning of the day before dosing (Day -1) and will remain resident for at least 7 days (168 hours) post-dose. Once the mass balance cumulative recovery >90% has been achieved or <1% of the dose administered has been collected in urine and faeces within in two consecutive days, subjects will be permitted to leave the clinical unit. Subjects may remain resident in the clinical unit for a maximum of 10 days post-dose.

A follow up phone call to ensure the ongoing wellbeing of the volunteers will be performed by a trained nurse or physician 5-7 days post discharge.

Figure 1 Study Flow Chart

≤21 days

Day -1 (am): Admission to clinical unit
Day 1 (am): Dosing
Day 1- 8: Sample collection
Day 8: Discharge (Subjects may remain resident until Day 10 where required)
A follow-up phone call to ensure the ongoing wellbeing of the volunteers will be performed by a trained nurse or physician 5-7 days post discharge.

Table 1 Time and Events Schedule

			Times after dosing (from start of infusion) (h)																								
	Screening ≤21 days	Admission	Pre-dose	0	0.25	0.5	1	1.25	1.5	2	2.5	3	4	5	6	8	12	16	24	36	48	60	72	96	120	144	168 (Discharge)	Follow-up call[i]
Informed Consent	X																											
Inclusion/ Exclusion criteria	X	X																										
Medical History	X	X																										
Height and Weight	X																											
Physical Examination	X	X																									X	
Haematology	X	X																									X	
Clinical Chemistry[a]	X	X																									X	
Serology	X																											
Urinalysis	X	X																									X	
Breath Test (carbon monoxide)	X	X																										
Alcohol Breath Test	X	X																										
Urine drug screen	X	X																										
IMP Administration[b]				X																								
PK Plasma			X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	

Table 1 Time and Events Schedule

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samples[c]																												
Whole blood samples[d]			X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
ECG	X		X				X												X								X	
Continuous ECG monitoring[e]			←---------→																									
Vital Signs[f]	X		X			X	X			X									X								X	
Urine samples[g]		←---------→																										
Faecal samples[h]		←---------→																										
Adverse Events		Assessed throughout the study period and at follow-up call																										

[a] Including creatinine clearance based on plasma creatinine
[b] The intravenous infusion will be administered over 60 minutes. Times from dose will be taken from the start of the infusion.
[c] Plasma samples will be collected for determination of parent drug, total radioactivity, metabolite profiling and identification
[d] Whole blood samples will be collected for determination of total radioactivity, metabolite profiling and identification
[e] Continuous ECG monitoring (lead II) from 5 minutes before start of the IV infusion until 5 minutes post completion of the infusion
[f] Body temperature will be performed at screening
[g] Urine samples will be collected for determination of parent drug, total radioactivity, and selected metabolite profiling. Urine collection periods: predose (-12 – 0h), 0-2, 2-4, 4-8, 8-12, 12-24, 24-48, 48-72, 72-96, 96-120, 120-144 and 144-168 h postdose. Sample collection may continue up to 240 h postdose where required.
[h] Faecal samples will be collected for determination of total radioactivity, and selected metabolite profiling. Faecal collection periods: predose (-24 – 0h), 0-24, 24-48, 48-72, 72-96, 96-120, 120-144 and 144-168 h postdose. Sample collection may continue up to 240 h postdose where required.
[i] A follow-up phone call to ensure the ongoing wellbeing of the volunteers will be performed by a trained nurse or physician 5-7 days post discharge.

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3.2 Rationale for study design, doses and control groups

This study will be a Phase I, open-label, single dose, single centre study, performed in six healthy male subjects aged 30-65 years. The age has been selected in accordance with the ARSAC notes for guidance. A single dose of [^{14}C]NXL104 will be administered as an IV infusion delivered over 60 minutes.

The number of subjects will be restricted to 6 to minimise the number of subjects exposed to radioactivity while being able to obtain the information required. Sample size is based on previous experience with mass balance studies.

Healthy subjects will be used in order to ensure compliance with study procedures and to avoid interference with the results from disease processes and other drugs. The inclusion and exclusion criteria are defined such that the healthy subjects selected will be known to be free of any significant illness when included in the study. The study will be conducted at a single centre to ensure consistency in study conduct and management of the healthy subjects and study data.

The 500 mg dose of NXL104 was selected for this study on the basis that this is currently considered the therapeutic dose of NXL104. There were no significant safety concerns at the time of the selection of this dose.

4. SUBJECT SELECTION CRITERIA

The Investigator should keep a record of subjects who entered pre-study screening in the subject screening log.

Each subject should meet all of the inclusion criteria and none of the exclusion criteria for this study. Under no circumstances can there be exceptions to this rule. Each subject's GP will be informed of their participation in the study.

Subjects will be recruited from the panel or by direct advertising to the public.

4.1 Inclusion criteria

For inclusion in the study subjects should fulfil the following criteria:

1. Provision of informed consent prior to any study specific procedures.

2. Healthy male subjects aged 30-65 years inclusive.

3. Male subjects should be willing to use an adequate method of contraception (as defined in Section 5.1) from the day of dosing until 3 months after dosing with the investigational product.

4. Have a body mass index (BMI) between 18 and 32 kg/m^2 inclusive and weigh at least 50 kg and no more than 100 kg.

5. Clinically normal physical examination and laboratory findings as judged by the investigator, including negative test results for drug-of- abuse, alcohol, CO breath test and negative test results for Hepatitis B surface antigen, antibodies to Hepatitis C virus and antibodies to HIV at the Screening Visit.

6. Subjects must have at least one bowel movement per day.

7. Subjects must be willing and able to communicate and participate in the whole study.

4.2 Exclusion criteria

Subjects should not enter the study if any of the following exclusion criteria are fulfilled:

1. Involvement in the planning and/or conduct of the study (applies to both AstraZeneca staff and/or staff at the study site).

2. Previous enrolment or randomisation in the present study.

3. Any clinically significant disease or disorder (e.g. cardiovascular, pulmonary, gastrointestinal, liver, renal, neurological, musculoskeletal, endocrine, metabolic, malignant, psychiatric, major physical impairment,) which, in the opinion of the investigator, may either put the subject at risk because of participation in the study, or influence the absorption, distribution, metabolism and excretion of drugs

4. Participation in another clinical study with an investigational product during the last 3 months

5. Any clinically relevant abnormal findings in physical examination, vital signs, clinical chemistry, haematology, urinalysis, which, in the opinion of the investigator, may put the subject at risk because of his participation in the study.

6. QTc > 450 ms or QT > 500 ms or other ECG abnormality making interpretation more difficult, as judged by the investigator, or a history of additional risk factors for Torsades de Points (eg heart failure, hypokalemia, family history of long QT syndrome), which in the opinion of the Investigator may put the volunteer at risk.

7. Any positive result on screening for serum hepatitis B surface antigen, hepatitis C antibody and human immunodeficiency virus (HIV).

8. History of any drug or alcohol abuse in the past 2 years.

9. Regular alcohol consumption in males >21 units per week (1 unit = ½ pint beer, 25 mL of 40% spirit or a 125 mL glass of wine).

10. Current smokers and those who have smoked within the last 12 months. A breath carbon monoxide reading of greater than 10 ppm at screening/admission.

11. Radiation exposure from clinical studies, including that from the present study, excluding background radiation but including diagnostic X-rays and other medical exposures, exceeding 5 mSv in the last twelve months or 10 mSv in the last five years. No occupationally exposed worker, as defined in the Ionising Radiation Regulations 1999, shall participate in the study.

12. Positive drugs of abuse or alcohol breath test at screening/admission.

13. Serious adverse reaction or serious hypersensitivity to any drug or the formulation excipients.

14. Presence or history of allergy requiring treatment. Hayfever is allowed unless it is active; or has required treatment within the previous 2 months.

15. Donation or loss of greater than 400 mL of blood within the previous three months.

16. Subjects receiving prohibited medication as described in Section 5.1.

17. Planned in-patient surgery, dental procedure or hospitalisation during the study.

18. Subjects who, in the opinion of the Investigator, should not participate in the study for any other reason.

Procedures for withdrawal of incorrectly enrolled subjects see Section 5.9.

5. STUDY CONDUCT

5.1 Restrictions during the study

The following additional restrictions will be in place for the duration of the study:

1. Subjects must abstain from alcohol during the 24 h prior to admission and for the duration of the study period.

2. Subjects must not smoke from admission to the clinical unit and for the duration of the study period.

3. Subjects must not drink liquids or eat foods containing cranberry, caffeine or other xanthines from 24 h prior to admission and for the duration of the study period.

4. Subjects should refrain from eating foods containing poppy seeds for 48 h before admission and for the duration of the study period;

5. Subjects should abstain from consuming grapefruit, grapefruit juice, or orange marmalade (made with Seville oranges) beginning 7 days before Day 1 dosing Visit 2 and for the duration of the study period;.

6. Subjects must not take part in any unaccustomed strenuous exercise from the 72 h before the screening visit and then from 72 h prior to admission and for the duration of the study period;

7. Male subjects must use appropriate contraception (i.e. condoms) from the time of taking the dose of the study drug until 3 months after receipt of the dose of the IP. Sexual intercourse with pregnant partners should also be avoided unless condoms are used. In addition to the use of condoms, female partners of male subjects should use additional contraception from the time of dosing until 3 months after dose. Acceptable methods used by female partners include the oral contraceptive pill, hormonal implants, intra-uterine devices (IUDs) or diaphragms. Subjects must not donate sperm from the time of taking the dose of study drug until 3 months after dosing.

8. Abstain from taking any prescribed or non-prescribed medication (including vitamins, herbal remedies and mineral supplements) from at least 14 days prior to commencing Day 1 dosing Visit 2 until the Follow-up Visit. Paracetamol up to a maximum daily dose of 4 grams is permitted; however the investigator should be informed so this can be documented.

9. Abstain from taking St John's Wort from at least three weeks prior to commencing Day 1 dosing Visit 2 and for the duration of the study period;

10. Eat standardised meals during the residential periods in the clinical unit.

11. During the residential periods only decaffeinated beverages will be served in the clinical unit.

12. Fast from at least 10 hours before planned start of IV dosing, and for 4 hours (except water for thirst) prior to collection of safety samples being taken at any time during the study. A moderate amount of water is allowed up to one hour prior to dosing and may be resumed one hour after completion of dosing (end of infusion). A meal can be given 4 hours after dosing.

5.2 Subject enrolment

The Principal Investigator will:

1. Obtain signed informed consent from the potential subject before any study specific procedures are performed.

2. Assign potential subject a unique enrolment number (E-code). Numbers will be allocated in descending order beginning with "E0001999".

3. Determine subject eligibility. See Sections 4.1 and 4.2

4. At Visit 2 assign eligible healthy subjects a unique treatment number (subject number),

If a subject withdraws from participation in the study, then his enrolment/subject number cannot be reused.

5.2.1 Procedures for randomisation

The study will be an open label, non-controlled study of a single IV dose of [^{14}C]NXL104. Subjects will not be randomised but eligible subjects will be assigned a treatment number (subject number) prior to dosing. Numbers will be allocated in ascending order beginning with "101".

A treatment allocation list will be produced prior to dosing, which will dictate the treatments that should be administered to each subject. The treatment allocation list will be filed in the investigator site file."

5.3 Procedures for handling subjects incorrectly enrolled or initiated on investigational product

Subjects who fail to meet the inclusion/exclusion criteria should not, under any circumstances, be enrolled or receive study medication. There can be no exceptions to this rule.

Where subjects that do not meet the selection criteria are incorrectly enrolled in the study but have not initiated treatment, these subjects should be withdrawn from the study.

Where subjects that do not meet the selection criteria are incorrectly dosed in the study, the subjects in question should be withdrawn from the study.

The AstraZeneca Study Delivery Team Physician is to ensure all such decisions are appropriately documented. In situations where an agreement cannot be reached, the subject should have their study therapy stopped.

5.4 Blinding and procedures for unblinding the study (Not applicable)

5.5 Treatments

5.5.1 Identity of investigational product(s)

Table 2 Identity of investigational product

Investigational product	Dosage form and strength	Manufacturer
NXL104	500 mg IV infusion containing NMT 300μCi (11.1 MBq)[^{14}C]	

The [^{14}C]NXL104 drug substance will be manufactured by and shipped to for manufacture of the IV solution. All manufactured dosage forms will be prepared in accordance with the Investigational Medicinal Product Dossier and the approved Batch Manufacturing Record. The final QP release will be performed by .

5.5.2 Doses and treatment regimens

Dosing is planned to commence on the morning of Day 1 at approximately 08:00, following an overnight fast of at least 10 hours. The exact time of dosing will be decided based on logistics and will be documented in the source workbook.

Each subject will receive a single IV infusion administration containing approximately 500 mg NXL104 in approximately 100 mL 0.9% normal saline over 60 minutes and a target dose of not more than 300 μCi (11.1 MBq) on one occasion. Times from dose will be taken from the start of the infusion.

The infusion will be in the opposite arm used for PK blood sampling. To assess tolerability of the IV administration, the first subject will be dosed at 08:00. The second subject will be dosed approximately 30 minutes after the first subject. Dosing for all subsequent subjects will be staggered by at least 15 minutes.

5.5.3 Labelling

Labels will be prepared in accordance with Good Manufacturing Practice (GMP) and local regulatory guidelines. The labels will fulfil GMP Annex 13 requirements for labelling. Label text will be translated into local language.

5.5.4 Storage

All investigational products will be kept in a secure place under appropriate storage conditions according to the product labels. The storage location of the study drugs will be locked and only accessible to authorised study site personnel.

5.6 Concomitant and post-study treatment(s)

Apart from paracetamol (up to a maximum daily dose of 4 g) no concomitant medication or therapy will be allowed. The subjects should be instructed that no other medication is allowed including herbal remedies, vitamin supplements and over-the-counter products without the consent of the Investigator.

Other medication, which is considered necessary for the subject's safety and well being, may be given at the discretion of the Principal Investigator and recorded in the appropriate sections of the source workbook.

5.7 Treatment compliance

The administration of all study drugs (including investigational products) should be recorded in the appropriate sections of the source workbook.

Treatment compliance will be assured by the supervised administration of the investigational product by the Principal Investigator or delegate. The dose, date and time of administration of the investigational product will be recorded and checked by the monitor at monitoring visits.

5.7.1 Accountability

The study drug provided for this study will be used only as directed in the study protocol.

Detailed records will be kept of all clinical supplies administered to study subjects, or disposed of by Any damaged or otherwise unusable supplies will be retained for inspection by the Sponsor's study monitor before return or disposal.

The monitor will account for all study drugs received at the site. Study personnel will arrange for the appropriate destruction or return of all unused study drugs at the Sponsors request. Certificates of delivery return or destruction will be filed accordingly.

The Investigator is responsible for maintaining study drug accountability at site.

5.8 Discontinuation of investigational product

Subjects may be discontinued from investigational product (IP) in the following situations:

- Subject decision. The subject is at any time free to discontinue treatment, without prejudice to further treatment
- Adverse Event
- Severe non-compliance to study protocol
- Development of any study specific criteria for discontinuation
- Concurrent illness or requirement for prohibited medication

- A the discretion of the Principal Investigator

A subject who has been dosed and who withdraws during the course of a study day will normally be expected to remain at the site until the planned end of the study day, with any safety evaluations being made at intervals as described in the clinical protocol. Subjects may be allowed to leave the clinical unit earlier if no safety evaluations are required and the drug taken is not considered to pose a risk, at the discretion of the Investigator.

5.8.1 Procedures for discontinuation of a subject from investigational product

A subject that decides to discontinue taking the investigational product will always be asked about the reason(s) and the presence of any adverse events. If possible, they will be seen and assessed by an investigator(s). Adverse events will be followed up (See Sections 6.3.3 and 6.3.4).

If a subject is withdrawn from study, see Section 5.9.

5.9 Withdrawal from study

Subjects are at any time free to withdraw from study (investigational product and assessments), without prejudice to further treatment (withdrawal of consent). Such subjects will always be asked about the reason(s) and the presence of any adverse events. If possible, they will be seen and assessed by an investigator. Adverse events will be followed up (See Sections 6.3.3 and 6.3.4).

Subjects who are withdrawn for non-drug related adverse events may be replaced following discussion between the Principal Investigator and AstraZeneca to ensure a minimum number of 4 evaluable subjects at the end of the clinical study.

Subjects withdrawn due to a drug related adverse event will not be replaced.

6. COLLECTION OF STUDY VARIABLES

The study assessments are described in the sections below and the timing of these assessments are detailed in the Time and Events Schedule (see Table 1).

It is important that PK sampling occurs as close as possible to scheduled time. There are times where a clinical protocol requires more than one procedure to be completed at the same time point. In these instances the following will apply:

- Pharmacokinetic samples will take precedence over other procedures and will therefore be collected at the nominal time;
- Vital signs will be recorded a within +/- 10 minutes prior to the nominal time;
- ECG recordings will be taken within +/- of 15 minutes prior to the nominal time.

Where a clinical protocol specifies other procedures to be performed (e.g. urine collection), the order of procedures will be determined at study set-up by the responsible nurse in discussion with the Project Manager, who will write a file note following discussion with the Principal Investigator and the Sponsor.

All safety assessments will be timed and performed relative to the start of the intravenous infusion.

6.1 Recording of data

The Principal Investigator will ensure that data are recorded on the paper source workbooks/ electronic CRFs (prepared by They will ensure the accuracy, completeness, legibility and timelines of the data recorded, for data queries and all required reports according to any instructions provided.

6.2 Data collection and enrolment

At enrolment (Visit 1), each potential subject will provide informed consent prior to starting any study-specific procedures.

Demographic data and other characteristics will be recorded and will include date of birth, gender, race, alcohol consumption, smoking history, and other parameters as detailed in the time and events schedule.

Each subject will undergo screening within ≤21 days prior to admission to confirm eligibility. This will consist of:

- Informed consent
- Recording of personal data
- A detailed medical and surgical history with a review of the inclusion and exclusion criteria with the subject
- A physical examination, including height and weight
- Vital signs- resting supine blood pressure and pulse (including body temperature)
- A resting 12-lead paper electrocardiogram (ECG)
- Breath carbon monoxide measurement
- Blood samples for routine clinical chemistry, haematology and screen for hepatitis B surface antigen, antibodies to hepatitis C virus and antibodies to HIV
- Urine samples for routine urinalysis and drugs of abuse screen

If the start of the study is delayed for any reason so that the interval between screening and first dose exceeds 21 days, all or part of the screening procedures may be repeated at the discretion of the Investigator.

If a laboratory value is outside the normal range, a repeat blood sample may be obtained at the discretion of the Investigator. If the results of the repeat test are normal or not clinically significant the volunteer may be accepted into the study.

6.3 Safety

The Principal Investigator is responsible for ensuring that all staff involved in the study is familiar with the content of this section.

6.3.1 Definition of adverse events

An adverse event is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product. An undesirable medical condition can be symptoms (e.g., nausea, chest pain), signs (e.g., tachycardia, enlarged liver) or the abnormal results of an investigation (e.g., laboratory findings, electrocardiogram). In clinical studies, an AE can include an undesirable medical condition occurring at any time, including run-in or washout periods, even if no study treatment has been administered.

The term AE is used to include both serious and non-serious AEs.

6.3.2 Definitions of serious adverse event

A serious adverse event is an AE occurring during any study phase (i.e., run-in, treatment, washout, follow-up), that fulfils one or more of the following criteria:

- Results in death
- Is immediately life-threatening
- Requires in-patient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions
- Is a congenital abnormality or birth defect
- Is an important medical event that may jeopardise the subject or may require medical intervention to prevent one of the outcomes listed above.

For further guidance on the definition of a SAE, see Appendix B to the Clinical Study Protocol.

6.3.3 Recording of adverse events

Time period for collection of adverse events

Adverse Events will be collected from time of signature of informed consent, throughout the treatment period until the subjects are discharged from the study.

Follow-up of unresolved adverse events

Any AEs that are unresolved at the subject's last visit in the study are followed up by the Principal Investigator for as long as medically indicated, but without further recording in the source workbook. AstraZeneca retains the right to request additional information for any subject with ongoing AE(s)/SAE(s) at the end of the study, if judged necessary.

Variables

The following variables will be collected for each AE;

- AE (verbatim)
- The date and time when the AE started and stopped
- Intensity
- Whether the AE is serious or not
- Principal Investigator causality rating against the Investigational Product (yes or no)
- Action taken with regard to investigational product
- AE caused subject's withdrawal from study (yes or no)
- Outcome.

In addition, the following variables will be collected for SAEs:

- Date AE met criteria for serious AE
- Date Investigator became aware of serious AE
- AE is serious due to
- Date of hospitalisation
- Date of discharge
- Probable cause of death
- Date of death

- Autopsy performed
- Causality assessment in relation to Study procedure(s)
- Causality assessment in relation to Other medication
- Causality assessment in relation to Additional Study Drug
- Description of AE.

The following intensity ratings will be used and a new AE will be recorded if intensity changes:

1. mild (awareness of sign or symptom, but easily tolerated)
2. moderate (discomfort sufficient to cause interference with normal activities)
3. severe (incapacitating, with inability to perform normal activities)

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria in Section 6.3.2. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not a SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke but would be a SAE.

Causality collection

The Investigator will assess causal relationship between Investigational Product and each Adverse Event, and answer 'yes' or 'no' to the question 'Do you consider that there is a reasonable possibility that the event may have been caused by the investigational product?'

For SAEs causal relationship will also be assessed for other medication and study procedures. Note that for SAEs that could be associated with any study procedure the causal relationship is implied as 'yes'.

A guide to the interpretation of the causality question is found in Appendix B to the Clinical Study Protocol.

Adverse Events based on signs and symptoms

All AEs spontaneously reported by the subject or reported in response to the open question from the study personnel, or revealed by observation will be collected and recorded in the source workbook. When collecting AEs, the recording of diagnoses is preferred (when possible) to recording a list of signs and symptoms. However, if a diagnosis is known and there are other signs or symptoms that are not generally part of the diagnosis, the diagnosis and each sign or symptom will be recorded separately.

Adverse Events based on examinations and tests

The results from protocol mandated laboratory tests and vital signs will be summarised in the clinical study report. Deterioration as compared to baseline in protocol-mandated laboratory values, vital signs, ECGs should therefore only be reported as AEs if they fulfil any of the SAE criteria or are the reason for discontinuation of treatment with the investigational product.

If deterioration in a laboratory value/vital sign is associated with clinical signs and symptoms, the sign or symptom will be reported as an AE and the associated laboratory result/vital sign will be considered as additional information. Wherever possible the reporting Principal Investigator uses the clinical, rather than the laboratory term (e.g., anaemia versus low haemoglobin value). In the absence of clinical signs or symptoms, clinically relevant deteriorations in non-mandated parameters should be reported as AE(s).

Cases where a subject shows an AST or ALT ≥3xULN or total bilirubin ≥2xULN may need to be reported as SAEs, please refer to Appendix D 'Actions required in cases of combined increase of Aminotransferase and Total Bilirubin – Hy's Law', for further instructions.

Deterioration of a laboratory value, which is unequivocally due to disease progression, should not be reported as an AE/SAE.

Any new or aggravated clinically relevant abnormal medical finding at a physical examination as compared with the baseline assessment will be reported as an AE.

6.3.4 Reporting of serious adverse events

All SAEs have to be reported, whether or not considered causally related to the investigational product, or to the study procedure(s). All SAEs will be recorded in the source workbook and the AstraZeneca SAE form will be completed.

If any SAE occurs in the course of the study, then investigators or other site personnel inform appropriate AstraZeneca representatives within one day i.e., immediately but **no later than the end of the next business day** of when he or she becomes aware of it.

The designated AstraZeneca representative works with the investigator to ensure that all the necessary information is provided to the AstraZeneca Patient Safety data entry site **within one calendar day** of initial receipt for fatal and life threatening events **and within five calendar days** of initial receipt for all other SAEs.

For fatal or life-threatening adverse events where important or relevant information is missing, active follow-up is undertaken immediately. Investigators or other site personnel inform AstraZeneca representatives of any follow-up information on a previously reported SAE within one calendar day i.e., immediately but **no later than the end of the next business day** of when he or she becomes aware of it.

The reference document for definition of expectedness/listedness is the IB for the AstraZeneca drug CAZ104 and the EU Summary of Product Characteristics (SPC) for NXL104 and CAZ.

Investigators or other site personnel send relevant source workbook pages by fax to the designated AstraZeneca representative

Follow-up information on SAEs should also be reported to AstraZeneca by the investigator(s) within the same time frames. If a non-serious AE becomes serious, this and other relevant follow-up information should also be provided to AstraZeneca within 1 day as described above.

The following information is required in the initial SAE report to AstraZeneca from the investigator(s); study code, site number, subject number, adverse event, seriousness, start date, and investigators causality assessment. The remaining detailed information should be sent to AstraZeneca as soon as it becomes available.

6.3.5 Reporting of Suspected Unexpected Serious Adverse Reactions (SUSARs)

It is the responsibility of AstraZeneca to determine whether a reported SAE fits the classification of a SUSAR and to notify the Principal Investigator of their decision as soon as possible.

In case a subject shows an AST or ALT ≥3ULN or total bilirubin≥2xULN please refer to Appendix D 'Actions required in cases of combined increase of Aminotransferase and Total Bilirubin – Hy's Law', for further instructions.

6.3.6 Fatal or life-threatening SUSARs

It is the responsibility of the AstraZeneca to report fatal or life-threatening SUSARs to the MHRA and EMA as soon as possible, but no later than 7 calendar days after they first became aware of the reaction.

For fatal or life threatening adverse drug reactions where important or relevant information is missing, active follow-up should be undertaken immediately and this information forwarded to AstraZeneca within one day.

The Principal Investigator is required to notify the EC of any SUSAR as soon as possible but no later than 7 calendar days after they first became aware of the reaction.

6.3.7 Other SUSARs

It is the responsibility of AstraZeneca to report other SUSARs to the MHRA as soon as possible, but no later than 15 calendar days after they first became aware of the reaction.

The Principal Investigator is required to notify the EC of any other SUSAR as soon as possible but no later than 15 calendar days after they first became aware of the reaction.

6.3.8 Laboratory safety assessment

Blood and urine samples for determination of clinical chemistry, haematology, and urinalysis will be taken at the times indicated in the Time and Events Schedule (See Table 1) Laboratory investigations will be performed at:

The following laboratory variables will be measured:

Clinical chemistry

Plasma (P) –Alanine Transferease (ALT, SGPT)

P-Albumin

P-Alkaline Phosphatase (ALP)

P-Aspartate Transferase (AST, SGOT)

P-Bilirubin (Direct) (and if Elevated, Conjugated Bilirubin)

P-Bilirubin (Total)

P-Calcium

P-Chloride

P-Creatinine (including creatinine clearance in plasma)

P-Glucose (Fasting)

P-Gamma Glutamyl Transpeptidase (GGTP)

P-Potassium

P-Phosphate (Inorganic)

P-Protein (Total)

P-Sodium

P-Urate (Uric Acid)

P-Urea

Urine Drugs of Abuse

U-Amphetamines

U-Barbiturates

U-Benzodiazepines

U-Cocaine

U-Marijuana/Cannabis

U-Methadone

U-Methamphetamine/Ecstasy

U-Morphine/Opiates

U-Phencyclidine

U-Tricyclic Antidepressants

Haematology

Blood (B)-Basophils
B-Complete Blood Count (CBC) with differentials

B-Eosinophils

B-Haematocrit (Packed Cell Volume-PCV)

B-Haemoglobin

B-Lymphocytes

B-Mean Corpuscular Haemoglobin (MCH)

B- Mean Corpuscular Haemoglobin Concentration (MCHC)

B-Mean Corpuscular Volume (MCV)

B-Monocytes

B-Neutrophils
B-Leukocyte (White Blood Cell count)

B-Platelet count

B-Red Blood Cell Count

Urinalysis

Urine (U)-Glucose

U-Bilirubin

U-Blood

U-Glucose

U-Urobilinogen

U-Ketones

U-Leukocytes

U-pH

U-Protein

U-Microscopy (At discretion of PI)

Serology

P-Hepatitis B Surface Antigen

P-Hepatitis C Antibody

P-HIV Antibody

An alcohol screen and breath carbon monoxide test will be performed at screening and on admission for Visit 2. If a subject tests positive to any of these screening tests he will be excluded from the study.

Laboratory values outside the reference limits are suspected to be of any clinical significance, as judged by the principal investigator and/or AstraZeneca, the sampling will be repeated. Subjects in whom suspected clinical significance is confirmed will either not be included or if already dosed will be followed until normalisation or for as long as the investigator considers necessary. Additional laboratory variables may be performed for safety reasons if judged appropriate by the investigator.

Laboratory data are to be considered for determining potential Hy's law cases during the time subjects are resident within the clinical unit. Subjects with elevated AST, ALT and/or total bilirubin should be followed up until resolution or recover, or until the study investigator considers that no further information will be forthcoming.

In cases where a subject shows an AST or ALT ≥3xULN or total bilirubin ≥2xULN, please refer to Appendix D 'Actions required in cases of combined increase of Aminotransferase and Total Bilirubin – Hy's Law', for further instructions.

6.3.9 Physical examination

A complete physical examination will be performed at the times indicated in the Time and Events Schedule (see Table 1). The examination will include an assessment of the following: general appearance, respiratory, cardiovascular, abdomen, skin, head and neck (including ears, eyes, nose and throat), lymph nodes, thyroid, abdomen, musculo-skeletal (including spine and extremities) and neurological systems.

Height will be measured in centimetres and weight in kilograms. Measurements should be taken without shoes, heavy clothing (e.g. coats) and the same scale used for all measurements. BMI will be calculated from the height and weight.

6.3.10 ECG

6.3.10.1 Resting 12-lead ECG

Resting 12-lead ECGs will be performed after 5 minutes supine at the times indicated in the Time and Events Schedule (see Table 1). Paper ECGs will be reviewed by the Principal Investigator or delegate and evaluated in his/her opinion, 'Normal' or 'Abnormal clinically/not clinically significant' and stored with the source workbook. If the QTc is considered prolonged a repeat paper ECG will be recorded to confirm if the change is still present.

6.3.10.2 Continuous ECG monitoring

Continuous ECG monitoring will be performed from -5 minutes before the start of infusion to 5 minutes post-completion of the infusion. This will be reviewed in real time, not recorded

but will be documented as normal or otherwise with any abnormal reading documented within the case report form.

6.3.11 Vital signs

6.3.11.1 Pulse and blood pressure

At each scheduled timepoint, blood pressure and pulse rate will be obtained after 5 minutes supine rest using a semi-automatic blood pressure recording device using the appropriate cuff size.

For timings of assessments refer to Time and Events Schedule (see Table 1). Additional blood pressure/pulse assessments may be taken for safety at the discretion of the Principal Investigator or delegate.

6.3.11.2 Body temperature

Body temperature will be measured at screening in degrees Celsius using an automated thermometer.

Additional temperature assessments may be taken for safety at the discretion of the Principal Investigator. If a subject develops a temperature, i.e. greater than baseline values, it will be monitored by the unit staff, outside of the stated times, until it returns to normal.

6.4 Pharmacokinetics

The following section describes handling of samples for pharmacokinetics.

Venous blood samples will be withdrawn via an indwelling cannula or by venepuncture. The first 0.5 mL of blood withdrawn via the cannula will be discarded and the subsequent required volume will be withdrawn. The cannula will be kept clear by flushing with physiological saline immediately after sampling and at intervals between samples.

The acceptable deviation from the nominal blood sampling times is ±10% unless otherwise specified by Sponsor.

If subjects meet the discharge criteria (see Section 9.5) earlier than 168 h postdose, subjects will be discharged from the clinical unit and subsequent samples will not be taken.

For blood volume see Section 7.1.

6.4.1 Whole Blood and Plasma

6.4.1.1 Total Radioactivity Measurement

Total radioactivity measurements will be determined from whole blood and plasma samples at Details of the analysis will be documented in a separate Study Plan.

Blood samples (6 mL) for the measurement of total radioactivity in whole blood and plasma will be collected into a 6 mL sodium fluoride/potassium oxalate tube at the following times:

Predose and 0.25, 0.5, 1, 1.25, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 16, 24, 36, 48, 60, 72, 96, 120, 144 and 168 hours postdose.

Approximately 2 mL of the 6 mL blood sample will be transferred into a plain tube and stored at ca 4 °C until shipment. The remaining 4 mL of blood will be processed in accordance with the Laboratory manual until shipment.

The blood and plasma samples will be shipped on a daily basis to

6.4.1.2 Pharmacokinetic Measurement

Blood samples (4 mL) for the pharmacokinetic measurement of NXL104 will be collected into a 4 mL sodium fluoride/potassium oxalate tube at the following times:

Predose and 0.25, 0.5, 1, 1.25, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 16, 24, 36, 48, 60, 72, 96, 120, 144 and 168 hours postdose.

The samples will be processed to obtain plasma in accordance with the Laboratory manual.

The samples will be shipped to for analysis at the end of the collection period.

6.4.1.3 Metabolite Profiling and Identification

Blood samples (20 mL) for the metabolite profiling and identification in plasma will be collected into sodium fluoride/potassium oxalate tubes at the following times:

Predose and 0.5, 1, 2, 4, 8, 16 and 24 hours postdose.

The samples will be processed to obtain plasma in accordance with the Laboratory manual and stored at -80ºC until shipment to AstraZeneca for analysis.

6.4.2 Urine Samples

Urine will be collected in polypropylene containers at the following times:

Predose (-12-0hr) and then at 0–2, 2-4, 4–8, 8–12, 12–24, 24–48, 48–72, 72–96, 96–120, 120–144 and 144–168 hours postdose.

6.4.2.1 Total radioactivity

Samples will be stored at ca 4°C until shipment. The collected samples will be shipped on a daily basis to

At the end of each timepoint the samples will be combined per subject and the net weight recorded by

6.4.2.2 Pharmacokinetic Measurement

Urine sub-samples from each timepoint will be retained by in polypropylene containers in accordance with the Laboratory Manual.

The samples will be shipped to for analysis at the end of the collection period.

6.4.2.3 Metabolite Profiling and Identification

Urine sub-samples from each timepoint will be retained by in polypropylene containers in accordance with the Laboratory Manual.

The samples will be shipped to AstraZeneca for analysis at the end of the collection period.

6.4.3 Faecal samples

Faecal samples will be collected for determination of total radioactivity and metabolite profiling in faeces at the following timepoints:

Predose (-24–0), 0-24, 24–48, 48–72, 72–96, 96–120, 120–144 and 144–168 hours posdose.

Samples will be stored at ca -20°C until shipment. The samples will be shipped on a daily basis to

At the end of each timepoint the samples will be combined per subject and the net weight recorded. Faeces samples will be homogenised in approximately an equal weight of water. Faecal homogenate sub samples from each timepoint (2 x ca 50 g) will be retained separately in polypropylene containers and stored at ca -20°C until shipped to AstraZeneca (if required). Bulk samples will be retained and stored at ca -20°C until issue of the draft analytical report.

6.4.4 Determination of drug concentration

Samples for determination of NXL104 in plasma and urine will be analysed by on behalf AstraZeneca, using a validated liquid chromatography and mass-spectrometry method (LC-MS/MS).

Samples for determination of total radioactivity in whole blood, plasma, urine and faeces will be analysed by on behalf AstraZeneca, using a validated liquid scintillation counting (LSC) method.

Plasma, urine and faecal samples (if required) will be sent to AstraZeneca for metabolite profiling and characterisation.

6.5 Discharge from the study

Subjects will be resident in the clinic until 168 hours after dosing but may be discharged earlier if a mass balance cumulative recovery of > 90% has been achieved and/or if a mean of < 1% of the dose administered has been collected in urine and faeces within two separate,

consecutive 24 h periods. In this case, collection of all samples (blood, urine and faeces) will be stopped and the subject will undergo the discharge assessments.

If recovery of radioactivity is < 90% at the end of the collection period (168 hours post dose), subjects may be required to remain in the clinical unit up to 10 Days post dose for further collections to be obtained. If insufficient radioactivity has been recovered by 10 days post dose, subjects may be required to collect samples for an extended period at home, if necessary.

The last visit of the last subject to the clinic will be considered the end of the study.

Subjects will be permitted to leave the clinic providing that:

1. no AEs have been reported during the study visit; and

2. the subject responds in the affirmative when asked if he is feeling well.

If any of these conditions is not met, then the subject will only be allowed to leave the clinical unit with the authorisation of the principal Investigator or appropriately qualified delegate.

6.6 Follow-up call

A follow up phone call to ensure the ongoing wellbeing of the volunteers will be performed by a trained nurse or physician 5-7 days post discharge.

7. BIOLOGICAL SAMPLING PROCEDURES

7.1 Volume of blood

The total volume of blood that will be drawn from each subject in this study is as follows:

Table 3 Volume of blood to be drawn from each subject

Assessment		Sample volume (mL)	No. of samples	Total volume (mL)
Safety	Clinical chemistry	5	3	15
	Haematology	2	3	6
	Serology	5	1	5
Pharmacokinetic	Parent	4	24	96
	Total radioactivity	6	24	144
	Metabolites	8	20	160
	Discard	0.5	24	
Total				**438**

The first 0.5 mL of blood withdrawn via the cannula will be discarded. The number of samples may be amended as deemed necessary. However, in this case, the total blood volume for each subject will not exceed 550 mL in a 4 week period.

7.2 Handling, storage and destruction of biological samples

The samples will be used up or disposed of after analyses or retained for further use as described here.

7.2.1 Pharmacokinetic samples

Samples will be disposed of after the clinical study report has been finalised, unless retained for future analyses, see below.

Key samples for investigation of metabolite identification and/or analysis will be retained by AstraZeneca, for a maximum of 10 years following the finalisation of the Clinical Study Report. The results from the investigation will not be reported in the Clinical Study Report but separately in an *in vivo* metabolism report.

7.3 Labelling and shipment of biohazard samples

The Principal Investigator will ensure that samples are labelled and shipped in accordance with the Laboratory Manual and the Biological Substance, Category B Regulations (materials containing or suspected to contain infectious substances that do not meet Category A criteria), see Appendix C 'IATA 6.2 Guidance Document'.

Any samples identified as Infectious Category A materials are not shipped and no further samples will be taken from the subject unless agreed with AstraZeneca and appropriate labelling, shipment and containment provisions are approved.

7.4 Chain of custody of biological samples

A full chain of custody is maintained for all samples throughout their lifecycle.

The Principal Investigator will keep full traceability of collected biological samples from the subjects while in storage at the centre until shipment or disposal (where appropriate).

The sample receiver will keep full traceability of the samples while in storage and during use until used or disposed of or until further shipment and will keep documentation of receipt of arrival.

AstraZeneca keeps oversight of the entire life cycle through internal procedures, monitoring of study sites and auditing of external laboratory providers.

Samples retained for further use are registered in the AstraZeneca biobank system during the entire life cycle.

7.5 Withdrawal of informed consent for donated biological samples

If a subject withdraws consent to the use of donated biological samples, the samples will be disposed of/destroyed, and the action documented. If samples are already analysed, AstraZeneca is not obliged to destroy the results of this research.

As collection of the biological samples is an integral part of the study, then the subject is withdrawn from further study participation.

The Principal Investigator:

- Ensures subjects' withdrawal of informed consent to the use of donated samples is notified immediately to AstraZeneca.
- Ensures that biological samples from that subject, if stored at the study site, are immediately identified, disposed of /destroyed, and the action documented
- Ensures the laboratory(ies) holding the samples is/are informed about the withdrawn consent immediately and that samples are disposed/destroyed, the action documented and the signed document returned to the study site
- Ensures that the subject and AstraZeneca are informed about the sample disposal.

ensures the central laboratory holding the samples is informed about the withdrawn consent immediately and that samples are disposed of/destroyed and the action documented and returned to the study site.

8. ETHICAL AND REGULATORY REQUIREMENTS

8.1 Ethical conduct of the study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/Good Clinical Practice, applicable regulatory requirements and the AstraZeneca policy on Bioethics and Human Biological Samples.

8.2 Subject data protection

The Informed Consent Form will incorporate (or, in some cases, be accompanied by a separate document incorporating) wording that complies with relevant data protection and privacy legislation.

8.3 Ethics and regulatory review

An Ethics Committee should approve the final study protocol, including the final version of the Informed Consent Form and any other written information and/or materials to be provided

to the subjects. The Principal Investigator will ensure the distribution of these documents to the applicable Ethics Committee, and to the study site staff.

The opinion of the Ethics Committee should be given in writing. The Principal Investigator should submit the written approval to AstraZeneca before enrolment of any subject into the study. The Ethics Committee should approve all advertising used to recruit subjects for the study.

AstraZeneca should approve any modifications to the Informed Consent Form that are needed to meet local requirements.

AstraZeneca will provide Regulatory Authorities, Ethics Committees and Principal Investigators with safety updates/reports according to local requirements, including SUSARs, where relevant.

The Principal Investigator is responsible for providing the Ethics Committee with reports of any serious and unexpected adverse drug reactions from any other study conducted with the investigational product. AstraZeneca will provide this information to the Principal Investigator so that he/she can meet these reporting requirements.

8.3.1 Administration of Radioactivity

Approval to administer the chosen dose of radioactivity will be sought from the Department of Health (UK) Administration of Radioactive Substances Advisory Committee (ARSAC) prior to commencing the study. Before submitting to the ARSAC and Ethics Committee, a summary of available nonclinical tissue distribution and excretion information on [^{14}C]NXL104 will be submitted to the Radiation Protection Division of the Health Protection Agency (HPA) for human dosimetry calculations in order to facilitate selection of the dose of radioactivity to be administered. The final report from the HPA will be included in the application to the ARSAC.

As part of the Protocol review process, the clinical protocol will be reviewed and approved by the ARSAC certificate holder.

ARSAC approval is given in the form of a certificate allowing the designated Certificate Holder to administer the approved dose. Copies of the ARSAC certificate will be submitted to the EC and the MHRA.

8.4 Informed consent

The Principal Investigator(s) at each centre will:

- Ensure each subject is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study
- Ensure each subject is notified that they are free to discontinue from the study at any time

- Ensure that each subject is given the opportunity to ask questions and allowed time to consider the information provided
- Ensure each subject provides signed and dated informed consent before conducting any procedure specifically for the study
- Ensure the original, signed Informed Consent Form(s) is/are stored in the Investigator's Study File
- Ensure a copy of the signed Informed Consent Form is given to the subject
- Ensure that any incentives for subjects who participate in the study as well as any provisions for subjects harmed as a consequence of study participation are described in the informed consent form that is approved by an Ethics Committee.

If any new information on the study medication becomes available which may influence the decision of the subject to continue the study, the investigator(s) should inform the subject of such information immediately, record this in a written form, and confirm with the subject if he or she wishes to continue the participation in the study. In addition, if the investigator(s) deem it necessary to revise the Informed Consent Form, they should revise it immediately (Refer to Section 9.5). The investigator(s) should re-explain the subjects using updated Informed Consent Form even if although the subjects have already been informed of the new information verbally. Written informed consent to continue participation in the study should be provided separately.

8.5 Changes to the protocol and informed consent form

Study procedures will not be changed without the mutual agreement of the Principal Investigator and AstraZeneca.

If there are any substantial changes to the study protocol, then these changes will be documented in a study protocol amendment and where required in a new version of the study protocol (Revised Clinical Study Protocol).

The amendment must be approved by the Ethics Committee, ARSAC certificate holder and if applicable, also the national regulatory authority, before implementation. Local requirements must be followed for amended protocols.

AstraZeneca will distribute any subsequent amendments and new versions of the protocol to each Principal Investigator(s). For distribution to Ethics Committee see Section 8.3.

If a protocol amendment requires a change to a centre's Informed Consent Form, AstraZeneca and the centre's Ethics Committee are to approve the revised Informed Consent Form before the revised form is used.

If local regulations require, any administrative change will be communicated to or approved by each Ethics Committee.

8.6 Audits and inspections

Authorised representatives of AstraZeneca, a regulatory authority, or an Ethics Committee may perform audits or inspections at the centre, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents, to determine whether these activities were conducted, and data were recorded, analysed, and accurately reported according to the protocol, Good Clinical Practice (GCP), guidelines of the International Conference on Harmonisation (ICH), and any applicable regulatory requirements. The investigator will contact AstraZeneca immediately if contacted by a regulatory agency about an inspection at the centre.

9. STUDY MANAGEMENT BY ASTRAZENECA

9.1 Pre-study activities

Before the first subject is entered into the study, it is necessary for a representative of AstraZeneca to visit the investigational study site to:

- Determine the adequacy of the facilities
- Determine availability of appropriate subjects for the study
- Discuss with the investigator(s) (and other personnel involved with the study) their responsibilities with regard to protocol adherence, and the responsibilities of AstraZeneca or its representatives. This will be documented in a Clinical Study Agreement between AstraZeneca and the investigator.

9.2 Training of study site personnel

Before the first subject is entered into the study, an AstraZeneca representative will review and discuss the requirements of the Clinical Study Protocol and related documents with the investigational staff and also train them in any study specific procedures.

The Principal Investigator will ensure that appropriate training relevant to the study is given to all of these staff, and that any new information relevant to the performance of this study is forwarded to the staff involved.

The Principal Investigator will maintain a record of all individuals involved in the study (medical, nursing and other staff).

9.3 Monitoring of the study

During the study, an AstraZeneca representative will have regular contacts with the study site, including visits to:

- Provide information and support to the investigator(s)

- Confirm that facilities remain acceptable
- Confirm that the investigational team is adhering to the protocol, that data are being accurately and timely recorded in the source workbooks, that biological samples are handled in accordance with the Laboratory Manual and that study drug accountability checks are being performed
- Perform source data verification (a comparison of the data in the eCRF with the subject's source workbook, and other records relevant to the study) including verification of informed consent of participating subjects. This will require direct access to all original records for each subject (e.g., clinic charts)
- Ensure withdrawal of informed consent to the use of the subject's biological samples is reported and biological samples are identified and disposed of/destroyed accordingly, and the action is documented, and reported to the subject.

The AstraZeneca representative will be available between visits if the investigator(s) or other staff at the centre needs information and advice about the study conduct.

9.3.1 Source data

The source workbook serves as the source for the following data:

- Physical examination results, blood pressure, pulse, temperature, height and weight
- Adverse events (subject notes may be source records)
- Concomitant medication (subject notes may be source records)
- Treatment administration
- Pharmacokinetic sample collection times
- Urine sample collection times
- Faecal sample collection times

A study-specific source data agreement will be finalised with the Sponsor prior to the start of the clinical phase of the study. The agreement will identify what data should be considered source data for this study.

9.4 Study agreements

The Principal Investigator should comply with all the terms, conditions, and obligations of the Clinical Study Agreement, or equivalent, for this study. In the event of any inconsistency between this Clinical Study Protocol and the Clinical Study Agreement, the terms of Clinical Study Protocol shall prevail with respect to the conduct of the study and the treatment of

subjects and in all other respects, not relating to study conduct or treatment of subjects, the terms of the Clinical Study Agreement shall prevail.

Agreements between AstraZeneca and the Principal Investigator should be in place before any study-related procedures can take place, or subjects are enrolled.

9.4.1 Archiving of study documents

The Investigator follows the principles outlined in the Clinical Study Agreement (CSA).

9.5 Study timetable and end of study

The end of the study is defined as 'the last visit of the last subject undergoing the study'.

The study is expected to start in and to end by .

The study may be terminated at the study centre if the study procedures are not being performed according to GCP, or if recruitment is slow. AstraZeneca may also terminate the entire study prematurely if concerns for safety arise within this study or in any other study with NXL104.

If AstraZeneca decides to prematurely terminate or suspend the study, the Principal Investigator, sub investigator, the head of the institution, and regulatory authorities should receive written notification of the reasons for the premature termination or suspension.

10. DATA MANAGEMENT BY

Data management will be performed by Clinical.

Full details of the data management process will be addressed in the Data Management Plan.

All completed workbooks will be issued to Data Management. All study data recorded in the workbooks will be entered into a validated clinical data management system (PhaseForward InForm V 5.0) with an audit trail to log all subsequent changes to the data. Following data entry, the data will be subjected to data consistency and validation checks, and the resulting output will be used to raise queries after reference to the workbooks. Queries will be raised and will be resolved with the assistance of staff.

Clinical chemistry and haematology data will be transferred electronically from the central laboratory. All demographic details and sample dates held by the central laboratory will be cross-referenced with the corresponding data on the database. All queries will be resolved with the assistance of the relevant staff.

Adverse events and medical/surgical history will be classified according to the terminology of the latest version the Medical Dictionary for Regulatory Activities (MedDRA). Medications will be classified according to the WHO Drug Dictionary.

The database will be closed after all queries have been resolved. The database will be locked following confirmation that the final error rate quality control (QC) checks are acceptable.

Audited plasma IP concentration data will be received electronically and cross-referenced with blood sampling data from the study database. The actual time of each blood sample will be calculated from the actual sample time and the time of dosing recorded in the study database, and merged with the concentration data.

The data collected through third party sources will be obtained and reconciled against study data.

11. EVALUATION AND CALCULATION OF VARIABLES BY CLINCAL

11.1 Calculation or derivation of safety variable(s)

11.1.1 Other significant adverse events (OAE)

During the evaluation of the AE data, an AstraZeneca medically qualified expert will review the list of AEs that were not reported as SAEs and DAEs. Based on the expert's judgement, significant adverse events of particular clinical importance may, after consultation with the Global Patient Safety Physician, be considered OAEs and reported as such in the Clinical Study Report. A similar review of laboratory/vital signs/ECG data will be performed for identification of OAEs.

Examples of these are marked haematological and other laboratory abnormalities, and certain events that lead to intervention (other than those already classified as serious), dose reduction or significant additional treatment.

11.2 Calculation or derivation of pharmacokinetic variables

The pharmacokinetic data for NXL104 in plasma, and total radioactivity in whole blood and plasma, provided by will be analysed by using

Plasma and whole blood concentration data will be tabulated and plotted for each subject for whom concentrations are quantifiable. Pharmacokinetic analysis of the concentration time data obtained will be performed using appropriate non-compartmental techniques to obtain estimates of the following pharmacokinetic parameters.

Where possible, the following PK parameters will be determined for NXL104 using the Plasma/urine/total radioactivity concentration data:

- C_{max}
- t_{max}
- $AUC_{0-\infty}$
- λz
- $t_{1/2}$
- MRT
- CLr
- CL_T
- Ae
- % excreted
- V_{ss}

Additional pharmacokinetic parameters may also be determined if deemed necessary to interpret the data. Full details will be provided within the Reporting and Analysis Plan (RAP).

PK parameters for metabolites of NXL104 will be calculated based on plasma, urine and faces concentration levels. Identification of major metabolites in plasma, urine and faeces will be performed where possible.

Mass Balance and cumulative recovery of total radioactivity in urine, faeces & total (urine and faeces) will be generated.

Determination of metabolic profiling and metabolite identification will be performed on relevant plasma, urine and faeces samples.

12. STATISTICAL METHODS AND SAMPLE SIZE DETERMINATION BY CLINICAL

12.1 Methods of statistical analyses

The Biometrics Department at t will produce all summary tables, figures and data listings for the analysis of safety and PK data using the statistical package SAS (v8.2 or a more recent version).

No formal statistical analysis will be performed on safety endpoints or PK endpoints for this study. Descriptive statistics including n, mean, standard deviation (SD), median, minimum, maximum and coefficient of variance (CV%) are considered adequate for a study of this type. Further details of descriptive analysis will be discussed in the RAP which will be prepared in accordance with SOPs. All summary tables, figures and data listings produced will be subjected to quality control.

Details of other considerations to be discussed in the RAP will include the following information:

1. Definition of analysis populations (e.g. safety and PK);

2. Reasonable detail covering the analyses and/or description of primary and secondary analyses and safety data;

3. Handling of missing data, unused or spurious data

4. Handling of data from withdrawn subjects.

12.2 Determination of sample size

This is an exploratory study to assess the mass balance recovery, metabolite profile and metabolite identification of [^{14}C]NXL104. No formal sample size calculation has been performed. A sample size of 6 subjects is considered appropriate to ensure a minimum number of 4 evaluable subjects at the end of the clinical study.

13. IMPORTANT MEDICAL PROCEDURES TO BE FOLLOWED BY THE INVESTIGATOR

13.1 Medical emergencies and AstraZeneca contacts

The Principal Investigator is responsible for ensuring that procedures and expertise are available to handle medical emergencies during the study. **A medical emergency usually constitutes an SAE and is to be reported as such, see Section 6.3.4**

In the case of a medical emergency the investigator may contact the Study Delivery Leader or Project Physician.

Name	Role in the study	Address & telephone number
Serious AE reporting	24-hour emergency cover at central R&D site.	

13.2 Overdose

Doses in excess of that planned according to the protocol are considered an overdose. In case of known or suspected overdose, symptomatic treatment as well as monitoring of vital functions should be performed, based on the judgment of the investigator.

- An overdose with associated AEs is recorded as the AE diagnosis/symptoms on the relevant AE modules in the source workbook.
- An overdose without associated symptoms is only reported on the source workbook.

If an overdose on an AstraZeneca study drug occurs in the course of the study, then investigators or other site personnel inform appropriate AstraZeneca representatives **within one day**, ie, immediately but no later than **the end of the next business day** of when he or she becomes aware of it.

The designated AstraZeneca representative works with the investigator to ensure that all relevant information is provided to the AstraZeneca Patient Safety data entry site.

For overdoses associated with SAE, standard reporting timelines apply, see Section 6.3.4. For other overdoses, reporting should be done within 30 days.

13.3 Pregnancy

All outcomes of pregnancy in partners of male subjects should be reported to AstraZeneca.

13.3.1 Paternal exposure

Male subjects should refrain from fathering a child or donating sperm during the study and for 3 months following the last dose.

Pregnancy of a subjects partner is not considered to be an adverse event. However, any conception occurring from the date of dosing until three months after dosing should be reported to AstraZeneca and followed up by for its outcome.

14. LIST OF REFERENCES

Cosgrove et al, 2004
Cosgrove SE, Kaye KS, Eliopoulos GM, Caremli Y. Health and economic outcomes of the emergence of third-generation cephalosporin resistance in Enterobacter species. Arch Intern Med. 2004;162:185–90

D'Agata 2004
D'Agata EM. Rapidly rising prevalence of nosocomial multidrug-resistant, Gram-negative bacilli: a 9-year surveillance study. Infect Control Hosp Epidemiol 2004;25(10):842-846.

Fraser et al, 2008
Fraser, SL, Arnett M, Sinave CP. Enterobacter Infections.
http://www.emedicine.com/med/topic678.htm. Updated 28 August 2008.

Fridkin et al, 1999
Fridkin SK, Gaynes RP: Antimicrobial resistance in intensive care units. Clin Chest Med. 1999;20:303-316

Gales et al, 2001
Gales AC, Jones RN, Turnidge J, Rennie R, Ramphal R. Characterization of Pseudomonas aeruginosa isolates: occurrence rates, antimicrobial susceptibility patterns, and molecular

typing in the global SENTRY Antimicrobial Surveillance Program, 1997–1999. Clin Infect Dis 2001;32(Suppl 2):S146–S155

Hiong U Go et al, 2004
Hiong U Go C, Burke A, Cunha, MD. Escherichia Coli Infections. www.emedicine.com Department of internal medicine, Winthrop-University Hospital Last Updated: June 27, 2004

Jacoby and Han 1996
Jacoby GA, Han P. Detection of extended-spectrum beta-lactamases in clinical isolates of Klebsiella pneumoniae and Escherichia coli. J Clin Microbiol 1996;34(4):908-911.

Kang et al, 2003
Kang CI, Kim SH, Kim HB, Park SW, Choe YJ, Oh MD et al. Pseudomonas aeruginosa bacteremia: risk factors for mortality and influence of delayed receipt of effective antimicrobial therapy on clinical outcome. Clin Infect Dis. 2003;37:745–51

Karlowsky et al; 2003a
Karlowsky JA, Jones ME, Thornsberry C, Friedland IR, Sahm DF. Trends in antimicrobial susceptibilities among Enterobacteriaceae isolates from hospitalized patients in the United States from 1998 to 2001. Antimicrob Agents Chemother. 2003;47:1672–80

Karlowsky et al, 2003b
Karlowsky JA, Draghi DC, Jones ME, Thornsberry C, Friedland IR, Sahm DF. Surveillance for antimicrobial susceptibility among clinical isolates of Pseudomonas aeruginosa and Acinetobacter baumannii from hospitalized patients in the United States, 1998 to 2001. Antimicrob Agents Chemother. 2003;47:1681–8

Karlowsky et al, 2004
Karlowsky JA, Jones ME, Draghi DC, Thornsberry C, Sahm DF and Volturo GA. Prevalence and antimicrobial susceptibilities of bacteria isolated from blood cultures of hospitalized patients in the United States in 2002. Annals of Clinical Microbiology and Antimicrobials. 2004;3:7

Lautenbach et al, 2001
Lautenbach E, Patel JB, Bilker WB, Edelstein PH, Fishman NO. Extended- spectrum β-lactamase-producing Escherichia coli and Klebsiella pneumoniae: risk factors for infection and impact of resistance on outcomes. Clin Infect Dis. 2001;32:1162–71

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Clinical Study Protocol
Appendix A

Drug Substance	NXL104
Study Code	D4280C00008
Edition Number	1
Date	
Protocol Dated	

Appendix A
Signatures

Appendix A
Drug Substance NXL104
Study Code D4280C00008
Edition Number 1
Date

ASTRAZENECA SIGNATURE(S)

An Open Label Single-Dose Study in Healthy Male Subjects Designed to Assess the Mass Balance Recovery, Metabolite profile and Metabolite Identification of [^{14}C]NXL104

This Clinical Study Protocol has been subjected to an internal AstraZeneca peer review.

I agree to the terms of this study protocol.

AstraZeneca Research and Development site representative

Day Month Year)

ASTRAZENECA SIGNATURE(S)

An Open Label Single-Dose Study in Healthy Male Subjects Designed to Assess the Mass Balance Recovery, Metabolite profile and Metabolite Identification of [14C]NXL104

This Clinical Study Protocol has been subjected to an internal AstraZeneca peer review.

I agree to the terms of this study protocol.

AstraZeneca Research and Development site representative

Appendix A
Drug Substance NXL104
Study Code D4280C00008
Edition Number 1
Date

SIGNATURE OF INTERNATIONAL CO-ORDINATING INVESTIGATOR

An Open Label Single-Dose Study in Healthy Male Subjects Designed to Assess the Mass Balance Recovery, Metabolite profile and Metabolite Identification of [14C]NXL104

This Clinical Study Protocol has been subjected to an internal AstraZeneca peer review.

I agree to the terms of this study protocol. I will conduct the study according to the procedures specified herein, and according to the principles of Good Clinical Practice and local regulations, and I ensure that all relevant site staff follows the instructions given in the latest version of the Laboratory Manual for Investigators.

Signature:

(Day Month Year)

AstraZeneca

Clinical Study Protocol Appendix B

Drug Substance	NXL104
Study Code	D4280C00008
Edition Number	1
Date	

Appendix B
Additional Safety Information

FURTHER GUIDANCE ON THE DEFINITION OF A SERIOUS ADVERSE EVENT (SAE)

Life threatening

'Life-threatening' means that the subject was at immediate risk of death from the AE as it occurred or it is suspected that use or continued use of the product would result in the subject's death. 'Life-threatening' does not mean that had an AE occurred in a more severe form it might have caused death (eg, hepatitis that resolved without hepatic failure).

Hospitalisation

Outpatient treatment in an emergency room is not in itself a serious AE, although the reasons for it may be (eg, bronchospasm, laryngeal oedema). Hospital admissions and/or surgical operations planned before or during a study are not considered AEs if the illness or disease existed before the subject was enrolled in the study, provided that it did not deteriorate in an unexpected way during the study.

Important medical event or medical intervention

Medical and scientific judgement should be exercised in deciding whether a case is serious in situations where important medical events may not be immediately life threatening or result in death, hospitalisation, disability or incapacity but may jeopardize the subject or may require medical intervention to prevent one or more outcomes listed in the definition of serious. These should usually be considered as serious.

Simply stopping the suspect drug does not mean that it is an important medical event; medical judgement must be used.

Examples of such events are:

- Angioedema not severe enough to require intubation but requiring iv hydrocortisone treatment
- Hepatotoxicity caused by paracetamol (acetaminophen) overdose requiring treatment with N-acetylcysteine
- Intensive treatment in an emergency room or at home for allergic bronchospasm
- Blood dyscrasias (eg, neutropenia or anaemia requiring blood transfusion, etc) or convulsions that do not result in hospitalisation
- Development of drug dependency or drug abuse.

A GUIDE TO INTERPRETING THE CAUSALITY QUESTION

The following factors should be considered when deciding if there is a "reasonable possibility" that an AE may have been caused by the drug.

- Time Course. Exposure to suspect drug. Has the subject actually received the suspect drug? Did the AE occur in a reasonable temporal relationship to the administration of the suspect drug?

- Consistency with known drug profile. Was the AE consistent with the previous knowledge of the suspect drug (pharmacology and toxicology) or drugs of the same pharmacological class? OR could the AE be anticipated from its pharmacological properties?

- Dechallenge experience. Did the AE resolve or improve on stopping or reducing the dose of the suspect drug?

- No alternative cause. The AE cannot be reasonably explained by another aetiology such as the underlying disease, other drugs, other host or environmental factors.

- Rechallenge experience. Did the AE reoccur if the suspected drug was reintroduced after having been stopped? AstraZeneca would not normally recommend or support a rechallenge.

- Laboratory tests. A specific laboratory investigation (if performed) has confirmed the relationship?

A "reasonable possibility" could be considered to exist for an AE where one or more of these factors exist.

In contrast, there would not be a "reasonable possibility" of causality if none of the above criteria apply or where there is evidence of exposure and a reasonable time course but any dechallenge (if performed) is negative or ambiguous or there is another more likely cause of the AE.

In difficult cases, other factors could be considered such as:

- Is this a recognised feature of overdose of the drug?

- Is there a known mechanism?

Ambiguous cases should be considered as being a "reasonable possibility" of a causal relationship unless further evidence becomes available to refute this. Causal relationship in cases where the disease under study has deteriorated due to lack of effect should be classified as no reasonable possibility.

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Clinical Study Protocol Appendix C	
Drug Substance	NXL104
Study Code	D4280C00008
Edition Number	1
Date	

Appendix C
International Airline Transportation Association (IATA) 6.2 Guidance Document

LABELLING AND SHIPMENT OF BIOHAZARD SAMPLES

International Airline Transportation Association (IATA) classifies biohazardous agents into 3 categories **(http://www.iata.org/whatwedo/cargo/dangerous_goods/infectious_substances.htm).** For transport purposes the classification of infectious substances according to risk groups was removed from the Dangerous Goods Regulations (DGR) in the 46th edition (2005). Infectious substances are now classified either as Category A, Category B or Exempt. There is no direct relationship between Risk Groups and categories A and B.

Category A Infectious Substances are infectious substances in a form that, when exposure to it occurs, is capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals. Category A pathogens are eg, Ebola, Lassa fever virus:

- are to be packed and shipped in accordance with IATA Instruction 602.

Category B Infectious Substances are infectious Substances that do not meet the criteria for inclusion in Category A. Category B pathogens are eg, Hepatitis A, B, C, D, and E viruses, Human immunodeficiency virus (HIV) types 1 and 2. They are assigned the following UN number and proper shipping name:

- UN 3373 – Biological Substance, Category B
- are to be packed in accordance with UN3373 and IATA 650

Exempt - all other materials with minimal risk of containing pathogens

- Clinical trial samples will fall into Category B or exempt under IATA regulations
- Clinical trial samples will routinely be packed and transported at ambient temperature in IATA 650 compliant packaging **(http://www.iata.org/whatwedo/cargo/dangerous_goods/infectious_substances.htm)**
- **Biological samples transported in dry ice require additional dangerous goods specification for the dry-ice content**
- IATA compliant courier and packaging materials should be used for packing and transportation and packing should be done by an IATA certified person, as applicable

- Samples routinely transported by road or rail are subject to local regulations which require that they are also packed and transported in a safe and appropriate way to contain any risk of infection or contamination by using approved couriers and packaging / containment materials at all times. The IATA 650 biological sample containment standards are encouraged wherever possible when road or rail transport is used.

AstraZeneca

Clinical Study Protocol Appendix D	
Drug Substance	NXL104
Study Code	D4280C00008
Edition Number	1
Date	

Appendix D

Actions Required in Cases of Combined Increase of Aminotransferase and Total Bilirubin - Hy's Law

1. ACTIONS REQUIRED IN CASES OF AST OR ALT ≥ 3X ULN OR TBL ≥ 2X ULN

The Investigator is responsible for, without delay, determining whether the subject meets potential Hy's law (PHL) criteria; Aspartate Aminotransferase (AST) or Alanine Aminotransferase (ALT) ≥ 3x Upper Limit of Normal (ULN) **and** Total Bilirubin (TBL) ≥ 2xULN at any point during the study, irrespective of Alkaline Phosphatase (ALP). The AST or ALT and total bilirubin values do not have to be elevated at the same visit or within any specified timeframe.

1.1 Identification

In cases of AST or ALT ≥ 3x ULN **or** TBL ≥ 2x ULN, please follow the instructions below.

When a subject has an AST or ALT ≥ 3xULN **or** TBL ≥ 2xULN at any visit, the central laboratory will immediately send an alert to the Investigator (also sent to the AstraZeneca representative)

If a subject is found to meet PHL criteria from a local laboratory sample:

- Repeat test with the central laboratory
- Complete the appropriate laboratory CRF modules with the original local laboratory test result.

1.2 Determination and Follow-up

1.2.1 Potential Hy's Law Criteria not met

If the subject **has not** had AST or ALT ≥ 3xULN **and TBL** ≥ 2xULN at any point in the study even if on different visits, irrespective of ALP

- Inform the AZ representative that the subject has not met PHL criteria
- Perform follow-up on subsequent laboratory results according to the guidance provided in the CSP.

1.2.2 Potential Hy's Law Criteria met

If the subject **has** had AST or ALT ≥ 3xULN **and TBL** ≥ 2xULN at any point in the study even if on different visits, irrespective of ALP:

- Notify the AZ representative who will then inform the central ST

The Study Physician (SP) contacts the Investigator, to provide guidance, discuss and agree an approach for the study subject's follow-up and the continuous review of data.

The Investigator:

- Follows the subject until liver biochemistry parameters and appropriate clinical symptoms and signs return to normal or baseline levels, or as long as medically indicated.
- Investigates the etiology of the event and perform diagnostic investigations as discussed with the SP
- Completes the Liver CRF Modules.
- If at any time (in consultation with the SP) the PHL case meets serious criteria, it should be reported as an SAE using standard reporting procedures.

1.3 Review and Assessment

No later than 3 weeks after the biochemistry abnormality was initially detected, the Study Physician contacts the Investigator in order to review available data and agree on whether there is an alternative explanation for the elevations in liver biochemistry other than Drug Induced Liver Injury (DILI) caused by the Investigational Medicinal Product (IMP).

For the purpose of this process a Hy's Law case is defined as:

Any subject with an increase in both Aspartate Aminotransferase (AST) or Alanine Aminotransferase (ALT) ≥ 3x Upper Limit of Normal (ULN) and Total Bilirubin (TBL) ≥ 2xULN, where no other reason can be found to explain the combination of increases, eg, elevated serum Alkaline Phosphatase (ALP) indicating cholestasis, viral hepatitis, another drug.

If there **is** an agreed alternative explanation for the AST or ALT **and TBL** elevations, a determination of whether the alternative explanation is an AE will be made and subsequently whether the AE meets the criteria for a SAE:

- If the alternative explanation is **not** an AE, record the alternative explanation on the appropriate CRF.
- If the alternative explanation is an AE/SAE, record the AE /SAE in the CRF accordingly and follow the AZ standard processes.

If it is agreed that there is **no** other explanation that would explain the AST or ALT and TBL elevations:

- Report an SAE (report term Hy's Law') according to AZ standard processes.
  - The 'Medically Important' serious criterion should be used if no other serious criteria apply
  - As there is no alternative explanation for the HL case, a causality assessment of related should be assigned.

If, there is an unavoidable delay, of over 3 weeks, in obtaining the information necessary to assess whether or not the case meets the criteria for a HL case, then it is assumed that there is no alternative explanation until such time as an informed decision can be made:

- Report an SAE (report term 'Potential Hy's Law') applying serious criteria and causality assessment as per above

2. REFERENCES

FDA Guidance for Industry (issued July 2009) 'Drug-induced liver injury: Premarketing clinical evaluation':

http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm064993.htm